Applying Novel Molecular Biological Tools in Detection and Prediction of Cyanobacterial Harmful Algal Blooms

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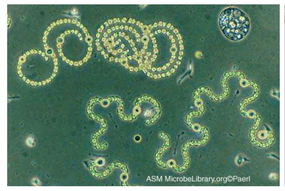
GOAL

Cyanobacterial harmful Algal Blooms (HABs) are a significant threat to water quality and potentially human health in the Great Lakes. In addition to contributing to hypoxia, degrading recreational value and disrupting food webs, HABs also may produce toxins that are detrimental to animal and human health. As a source of drinking and recreational water for as many as 40 million US and Canadian citizens, these toxins are of significant concern. Bloom toxicity can vary with environmental conditions and strain composition and the degree of toxicity may change over a bloom season. Clearly, the health effects of a HAB bloom are related to toxicity and early detection of the onset of a toxic bloom allows better management and protection of human health.

The goals of this work are to use novel molecular techniques to:

- Rapidly detect and quantify toxic cyanobacterial HAB species in Great Lakes waters using for drinking and recreation, with particular focus on early detection of toxic blooms.
- Identify ecological factors stimulating bloom toxicity, particularly the role of dreissenid mussels.

Microcystis aeruginosa



Anabaena sp.

Planktothrix sp.

Molecular Tools

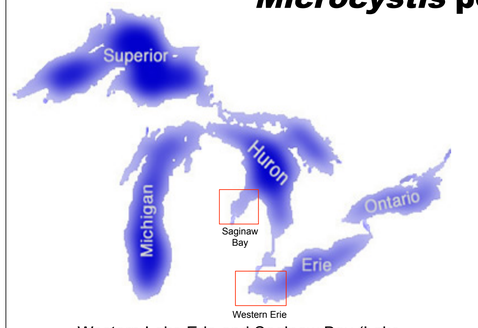
Toxic and nontoxic cyanobacterial HAB cells cannot be differentiated morphologically and thus traditional microscopy-based methods are not sufficient for identifying toxic blooms. Bloom toxicity can be assessed by detection of the toxin, but this does not provide information on which HAB species is responsible for its production, since multiple species can produce the same toxin. However, each HAB strain has a unique genetic signature and toxic strains can be identified based on the presence of the genes controlling toxin production.

The genetics behind toxin production is best understood for microcystin. Microcystin producing cyanobacteria, including *Microcystis*, contain a multiple gene operon, *mcy*, that controls microcystin synthesis (Fig. 1). Similar *mcy* operons are also found in the cyanobacterial HAB genera *Planktothrix* and *Anabaena,* also both commonly found in the eutrophic regions of the Great Lakes. The *mcyB* gene was chosen since it contains a higher degree of genetic variability, useful for differentiating between strains, but enough conservation to also design primers to detect all *Microcystis*. Both toxic and nontoxic *Microcystis* can be identified and quantified using a marker common to all cyanobacteria, the genes regulating phycocyanin production: *cpcB* and *cpcA*.

PCR, quantitative PCR and DNA sequencing targeting the *mcyB* and *cpc* genes were used to detect and quantify microcystin-producing HABs.

Fig. 1 *mcy* operon controlling microcystin production in *Microcystis*

Genetic variability in Great Lakes *Microcystis* populations



Western Lake Erie and Saginaw Bay (Lake Huron) are the regions most impacted by *Microcystis* blooms in the lower Great Lakes.

Microcystis bloom in western Lake Erie summer 2009.

Seasonal changes in bloom toxicity

Determined the percentage of the *Microcystis* population that is comprised of toxic cells throughout a western Erie bloom season (2 sites, summer 2009).

1) quantitative PCR of the *cpc* genes: total number of *Microcystis* cells.

2) quantitative PCR of the *mcyB* gene: number of toxic *Microcystis* cells.

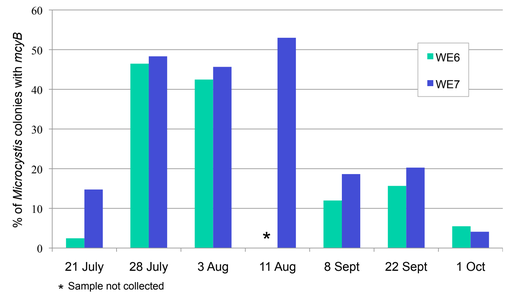


Fig. 2 Percentage of *Microcystis* bloom comprised of toxic cells during 2009 bloom season at two western Erie sites. Note that during the later stages of the bloom that there are more nontoxic cells present.

Spatial variability in population composition

Characterized the genetic variability in toxic *Microcystis* populations in western Erie and Saginaw Bay through DNA sequencing of *mcyB* from bloom samples.

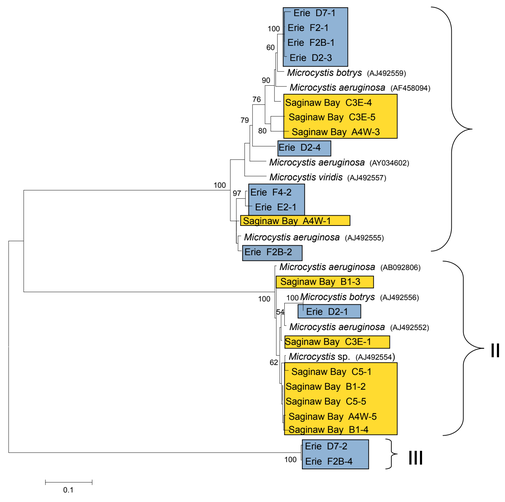


Fig. 3 Phylogenetic tree of *mcyB* sequences isolated from Saginaw Bay (highlighted in yellow) and western Lake Erie (highlighted in blue). Other *Microcystis* sequences are from GenBank and accession numbers are given in parentheses. Bootstrap values greater than 50% are indicated at each node. The three clusters indicate distinct populations of microcystin-producing cyanobacteria.

Cluster I – *Microcystis*
mix of Saginaw Bay and Erie strains

Cluster II – *Microcystis*
mostly Saginaw Bay strains

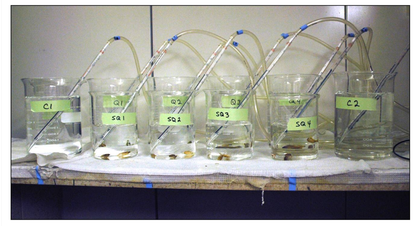
Cluster III – *Planktothrix*
all Erie strains

Dreissenid mussel experiments

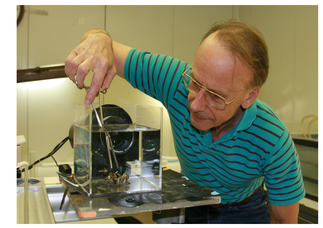
Impacts of dreissenid mussels on *Microcystis* bloom toxicity

Determine if dreissenid mussels differentiate between toxic and nontoxic cells during grazing:

1) 4 hr feeding experiment in which a single quagga mussel is exposed to a natural assemblage of *Microcystis* (collected from Saginaw Bay).
2) Control - natural seston alone and no quagga mussels.
3) *Microcystis* population sampled before and after feeding
   - collect two size fractions: >53 µm for whole colonies; < 53 µm for single cells.
4) Quantitative PCR for *cpc* and *mcyB* to determine if the quagga mussel feeding impacts the ratio of toxic:nontoxic cells in the seston.



Experiment set up and sampling for quagga mussel grazing experiment.



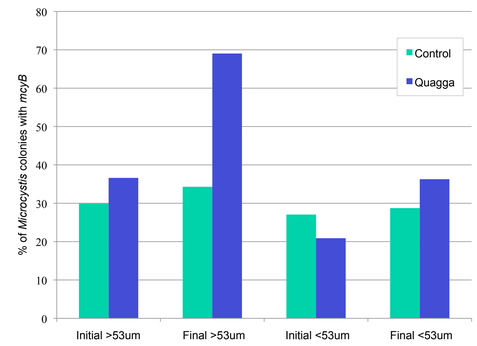


Figure 4. Percentage of toxic *Microcystis* cells present before and after 4 hr of quagga mussel feeding. Control beakers had no quagga mussels present. The % of toxic cells increased after mussel feeding in both the >53 µm and <53 µm fractions, while remaining the same in the control beakers.

Significant results

1) The percentage of the *Microcystis* population comprised of toxic strains changes over the course of the bloom season. Bloom toxicity peaks at mid-summer and moves towards more nontoxic strains during the end of the bloom.

2) There are genetically distinct populations of toxic *Microcystis* in Saginaw Bay and western Lake Erie, with one population residing mostly in Saginaw Bay (cluster II). *Planktothrix* (a microcystin-producing cyanobacteria) is primarily present in western Erie.

3) Dreissenid mussel grazing could select for toxic blooms by not consuming toxic strains.

Future work

1) Develop molecular probes for *mcyB* based on knowledge of genetic variability for use in future buoy-based sensors.

2) Use gene expression assays to determine if dreissenid mussels and/or other ecological factors upregulate microcystin production in cells.